

Claims

5

10

15

- 1. A method for determining a predisposition for a manifestation of an immune system related disease in an individual comprising determining in a biological sample isolated from said individual the presence or absence of a polymorphism within the amino acid sequence of the MASP-2 protein as identified in SEQ ID NO: 1 and/or within the amino acid sequence of the MAp-19 protein as identified in SEQ ID NO: 2, said polymorphism being a substitution, deletion and/or addition of least one amino acid residue.
 - 2. The method of claim 1, wherein the polymorphism a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 consisting of CUB1, EGF, CUB2, CCP1 and CCP2 domains.
 - 3. The method of claim 1, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 and/or MAp-19 consisting of CUB1, EGF, CUB2 domains.
- The method of claim 1, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 and/or MAp-19 consisting of CUB1.
- 5. The method according to any of the preceding claims, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid located within amino acid residues from position 80 to position 120 according to the amino acid sequences set forth in SEQ ID NO: 1 or 2.
 - The method according to any of the preceding claims, wherein the polymorphism being a substitution or deletion of Asp in position 105 according to the amino acid sequences set forth in SEQ ID NO: 1 or 2.
 - 7. The method of claim 6, wherein the substitution being Asp→Gly.
- 8. A method for determining a predisposition for a manifestation of an immune system related disease comprising determining the presence or absence of

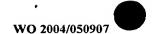
WO 2004/050907

15



polymorphism within the coding DNA sequence (SEQ ID NO: 3) of the human MASP-2 gene, said polymorphism being a substitution, addition or delition of at least one nucleotide within said coding DNA sequence.

- 9. The method of claim 8, wherein the DNA sequence comprising the polymorphism is a coding nucleic acid sequence for the proteins as defined in any of the claims 1-7.
- 10. The method of claim 8, wherein the polymorphism being a single nucleotide
 substitution/mutation A→G in position 359 corresponding to the sequence set forth in SEQ ID NO: 3.
 - 11. The method according to any of the claims 1-10, wherein the polymorphism is determined by isolating the MASP-2 and/or MAp-19 proteins from a biological sample collected from an individual and ascertaining the substitution/mutation in the amino acid sequence of said proteins by a method selected from the group comprising mass-spectroscopy methods, such as MALDI-TOF mass-spectroscopy, protein sequencing methods or immunoassays.
- 12. The method according to any of the claims 1-11 further comprising isolating the MBL-MASP or ficolin-MASP complexes from a biological sample collected from an individual and examining the activity of said complexes, said activity being determined as an ability the complexes to activate the C4 complement.
- 13. The method according to any of the claims 1-12 further comprising examining the protein composition of MBL or ficolin complexes in a biological sample collected from an individual.
- 14. The method according to any of the claims 1-13, wherein the predisposition to a manifestation of an immune system related disease is determined by the absence of the MASP-2 (SEQ ID NO: 1) and/or MAp19 (SEQ ID NO: 2) proteins in the MBL or ficolin complexes.
- 15. The method according to claims 8-10, wherein the presence or absence of the polymorphism is detected by hybridising a probe to a target nucleic acid



5

10

sequence comprising at least position 359 according to the SEQ ID NO: 3 or SEQ ID NO: 4 or the corresponding position of the complementary strand.

- 16. The method according to claim 15, wherein the probe is bound to a detectable label.
- 17. The method according to claim 16, wherein the label is selected from a group comprising fluorescent reporter groups, enzyme tags, chemiluminescent groups or radioisotopes.
- 18. The method according to claim 15, comprising the use of a capture probe for capturing a target nucleic acid sequence.
- 19. The method according to any of the preceding claims 15-18, comprising amplification of a nucleotide sequence comprising the polymorphism.
 - 20. The method according to claim 19, wherein amplification comprises use of a primer pair comprising SEQ ID NO: 5 and 6 or SEQ ID NO: 7 and 8.
- 21. The method according to claim 8, wherein the presence or absence of the polymorphism is detected by using isolation of a target nucleic acid from an individual said target nucleic acid comprising at least position 359 according to the sequence set forth in the SEQ ID NO: 3 or the corresponding position of the complementary strand and sequencing of said isolated target nucleic acid.
 - 22. The method according to any of the claims 8-21, further comprising assessing the alleles at nucleotide no. 359 according to the sequence set forth in SEQ ID NO: 3 in a target nucleotide sequence corresponding to SEQ ID NO: 3 or the complementary strand.
 - 23. An isolated oligonucleotide comprising at least 10 contiguous nucleotides of SEQ ID NO: 3 or the corresponding complementary strand, said nucleic acid sequence comprising the G allele in position 359 or the corresponding allele of the complementary strand.

25



5



- 24. The isolated oligonucleotide according to claim 23, comprising at least 15 contiguous nucleotides, more preferably at least 20 nucleotides.
- 25. An isolated polynucleotide sequence encoding the MASP-2 polypeptide having Gly at position 105 according to amino acid sequence set forth in SEQ ID NO: 1.
 - 26. An isolated polynucleotide sequence encoding the MAp-19 polypeptide having Gly at position 105 according to amino acid sequence set forth in SEQ ID NO: 2.
- 27. The isolated oligonucleotide or polynucleotide sequence according to any of the claims 23-26, wherein the nucleotides are selected from RNA, DNA, LNA, PNA monomers or chemically modified nucleotides capable of hybridising to a target sequence.
- 15 28. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1 or a fragment thereof, said polypeptide or said fragment comprising Gly in position 105 according to said sequence.
 - 29. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, said polypeptide or said fragment comprising Gly in position 105 according to said sequence.
 - 30. An isolated peptide fragment having a size in a range from 5 to 160 amino acids derived from the amino acid sequence set forth in SEQ ID NO: 1 comprising at least 5 amino acid contiguous sequence, said sequence corresponding to amino acid residues 100-105, 101-106, 102-107, 103-108, 104-109 and/or 105-110 of the sequence set forth in SEQ ID NO: 1.
 - 31. An isolated peptide fragment having a size in a range from 5 to 160 amino acids derived from the amino acid sequence set forth in SEQ ID NO: 1 comprising at least 5 amino acid contiguous sequence, said sequence corresponding to amino acid residues 100-105, 101-106, 102-107, 103-108, 104-109 and/or 105-110 of the sequence set forth in SEQ ID NO: 1, wherein Gly in position 105 of said sequence is substituted for Asp.

20

25



5

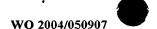
10

15

30



- 32. An isolated antibody capable of recognition of the MASP-2 and/or MAp-19 polypeptides or fragments thereof, said polypeptides and fragments comprising Gly in position 105 according to the SEQ ID NOS: 1 or 2, by selectively binding to an epitope comprising said Gly or selectively binding to an epitope created within said polypeptides or said fragments due to mutation of Asp→Gly in position 105 according to SEQ ID NOS:1 or 2.
- 33. An isolated antibody capable of recognition of the MASP-2 and MAp-19 polypeptides or fragments thereof by selectively binding to an epitope comprising Asp corresponding to position 105 of the sequence set forth in SEQ ID NOS: 1 or 2.
- 34. A kit for predicting an increased risk of a subject of developing an immunologic disease comprising at least one probe comprising a oligonucleotide sequence as defined by any of the claims 23-27 and/or at least one probe comprising at least one antibody as defined by claims 32 and 33, or a fragment of said antibody.
- 35. The kit according to claim 34, wherein the probe is linked to a detectable label.
- 36. The kit according to any of the claims 34-35, further comprising a set of primers for amplifying a region of the human MASP-2 gene said region comprising position 359 according to SEQ ID NO: 3 or the corresponding complementary strand.
- 37. A gene therapy vector for the treating pathologic conditions associated with low activity of MBL-pathway in a subject carrying the G allele in the position corresponding to nucleotide no 359 of the sequence identified in SEQ ID NO: 3.
 - 38. The gene therapy vector of claim 37, said vector comprising the sequence set forth in SEQ ID NO: 3, or a fragment thereof operably linked to a promoter sequence capable of directing the in vivo expression of MASP-2 encoded by SEQ ID NO: 3.
 - 39. A gene therapy vector for the treating therapeutic conditions associated with pathologically high activity of the MBL-pathway, said vector comprising the





nucleotide sequence identified as SEQ ID NO: 3, said sequence having substitution A→G in position 359, said sequence operably linked to a promoter sequence capable of directing the in vivo expression of MASP-2 having glycine residue in position 105 according the sequence set forth in SEQ ID NO: 1.

5

40. Use of a polypeptide comprising any of the amino acid sequences set forth in SEQ ID NOS: 1 or 2, or fragments thereof, said polypeptides or said fragments comprising Gly in position 105 of said sequences, for production of a medicament for the inhibition of activity of the lectin-complement pathway.

10

41. Use of a peptide fragment according to claim 40 for inhibition of activity of the lectin-complement pathway.

42. Use of a polypeptide comprising any of the amino acid sequences set forth in SEQ ID NOS: 1 or 2, or fragments thereof, said polypeptides or said fragments comprising the glycine residue in position 105 of said sequences for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.

- 43. Use of an oligonucleotide and/or polynucleotide as defined in any of the claims 25-28 for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.
- 44. Use of an antibody as defined in claim 33 or a fragment thereof for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.
- 45. The use according to claim 42-44, wherein the therapeutic conditions associated with pathologically high activity of MBL-complement pathway being an inflammatory disease, ischemia, apoptosis or atherosclerosis.
 - 46. A method of treatment of an individual having a predisposition to a manifestation of an immune system related disease comprising
- 35 I) identification a mutation in the MASP-2 gene of said individual and

II) administering to said individual an effective amount of a polypeptide comprising SEQ ID NO:1 and/or polypeptide comprising SEQ ID NO:2.